ÖGH Herpetozoa
Austrian Herpetological Society

Herpetozoa 33: 53–57 (2020)
DOI 10.3897/herpetozoa.33.e49768

Observations on the oviposition of *Blythia reticulata* (Blyth, 1854) with new distributional records from Mizoram State, NE India

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http://zoobank.org/034FCE06-FA00-4824-8A9A-AAEC278F1A5F

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Academic editor: Johannes Foufopoulos ♦ **Received** 31 December 2019 ♦ **Accepted** 13 April 2020 ♦ **Published** 7 May 2020

Abstract

The poorly known semi-fossorial snake *Blythia reticulata* is a small, oviparous, worm-eating species found in northeastern India and neighboring countries. Here we report on multiple new distribution localities that extend the known geographic range of the species. In addition, we provide new information on the reproductive biology of the species based on egg-laying behavior data from a captive gravid *B. reticulata* from Mizoram. The simultaneous presence of a second clutch of eight eggs in the oviduct of the female indicates the capacity of the species to exhibit multiple matings and egg clutches during a single reproductive season.

Key Words

Blyth's Reticulate Snake, Colubridae, eggs, fecundity, oviparous, reproduction, semi-fossorial

Introduction

Blythia Theobald 1868 is a colubrid snake genus comprising two extant species *Blythia reticulata* (Blyth, 1854) and *Blythia hmuifang* (Vogel, Lalremsanga & Vanlalhrima, 2017). *B. reticulata*, commonly known as Blyth's Reticulate Snake (Uetz 2019) or Iridescent Snake (Whitaker and Captain 2008; Das and Das 2017), is a small semi-fossorial snake, inhabiting evergreen forests at an elevation up to 1,040 m asl. (Whittaker and Captain 2008; Das 2012). The known distribution range of the species includes parts of Bangladesh, China, Myanmar and India (the states of Mizoram, Assam, Arunachal Pradesh, and Manipur (Das 2008; Purkayastha 2013; Das and Das 2017; Vogel et al. 2017). Little has been published about its natural history and reproduction, except that this oviparous snake lays clutches up to 6 eggs (Whitaker and Captain 2008). Little is also known about the ecology, life history, and genetics of *B. reticulata*. Given the paucity of information about this species, its conservation status is currently listed as 'Data Deficient' in the IUCN Red List (Wogan and Vogel 2012). Elucidating the reproductive biology of a species is particularly important both for understanding its general life history patterns and also for informing conservation management actions (Siegel and Ford 1987; Holycross and Goldberg 2001). Here, we contribute new details on the reproductive biology of wild-caught *B. reticulata*, as well as new distribution localities for the species from Mizoram State (NE India).

Materials and methods

Reproduction. The reproductive observations reported here come from a captive gravid *B. reticulata*. This individual was spotted and collected while crawling towards a

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homestead flower garden at Venghlui, Saitual town, Saitual District, Mizoram (23.674578N, 92.962397E; 1,130 m asl.; 8 Jul. 2019). The animal was subsequently transported to the facilities of the Developmental Biology and Herpetology Laboratory, at the Dept. of Zoology, Mizoram Univ. Aizawl. Environmental conditions were monitored with the help of a HTC-1 LCD Digital Hygrometer Thermometer with a temperature accuracy of ± 1 °C, and a humidity accuracy of ± 5%. Eggs laid on 10 Jul. 2019, at ca. 8:30 hrs were weighed using an electronic balance to the nearest 0.001 g (Gem20 High Precision Digital Milligram Scale, Smart Weigh). We measured the snout-vent length (SVL) and tail length (TL) to the nearest 1 mm using a flexible measuring tape. Scales were counted following the methodology of Dowling (1951) used for taxonomic confirmations. Both laid and oviductal egg sizes were measured using dial callipers (Mitutoyo, 506–671) to the nearest 0.1 mm. The animal was provisioned with both earthworms and insects but refused to feed, although it was observed drinking some water. For the incubation of eggs, an approx. 20 mm thick layer of vermiculite (mixed with water in a 2:1 ratio) was provided for bedding in a 150 mm × 150 mm × 80 mm polypropylene container covered with a perforated lid. Temperatures in the incubation box were maintained between 26.5 °C–28 °C with humidity between 85%–90%. Fresh leaves provided were occasionally sprayed with water for maintaining a proper humidity level. Photographs were taken with a digital camera (Canon PowerShot SX430 IS).

Distributional records. To prepare a distributional map of *B. reticulata* we collected geographical coordinates of specimen collection sites from Mizoram (India) using a portable GPS unit (Garmin Montana 650-GPS navigator). Field survey methodologies largely followed Doan (2003) and Manley et al. (2004) and specimens were collected using a Visual Encounter Survey (VES) approach by checking ground, bushes, leaf litter, underneath tree-bark, logs, rocks, around water bodies (e.g. streams, canals and tanks), as well as crevices of rocks and boulders, and also by digging into soil. Individuals were collected with the help of tongs or by hand. Collected specimens were kept in snake bags and later catalogued and deposited at the Departmental Museum of Zoology, Mizoram University (MZMU).

Results

Specimen collection. During the course of this study (2017–2019), we made collections of *B. reticulata* from five different localities in Mizoram State: Lungdai (MZMU 941, gravid ♀, SVL = 332 mm, TL = 34 mm, ventral scales = 134, subcaudal scales = 17; 23.881210N, 92.740351E; 1,180 m asl.; 12 May. 2017, ca. 16:00 hrs; Lalrengpuii Sailo leg.; collected from a footpath), Saitual (MZMU 1424, gravid ♀, SVL = 328 mm, TL = 35 mm, ventral scales = 140, subcaudal scales = 20; 23.674578N, 92.962397E; 1,130 m asl.; 8 Jul. 2019, ca. 8:30 hrs; BJ Hnamte leg.; collected from a vehicle road), Tlungvel (MZMU 1317, non-gravid ♀, SVL = 252 mm, TL = 28 mm, ventral scales = 137, subcaudal scales = 19; 23.605620N, 92.854482E; 1,120 m asl.; 15 Oct. 2018, ca. 9:30 hrs; Hmar Tlawmte Lalremsanga leg.; dug out from cultivation ground), Khawzawl (MZMU 935, ♂, SVL = 223 mm, TL = 34 mm, ventral scales = 124, subcaudal scales = 25; 23.529202N, 93.187097E; 1,280 m asl.; 20 Feb. 2017, ca. 9:00 hrs; Hrahsel Laltlanchhuaha leg.; unearthed from construction site), Tanhril (MZMU 960, non-gravid ♀, SVL = 209 mm, TL = 31 mm, ventral scales = 121, subcaudal scales = 22; 23.738542N, 92.673410E; 950 m asl.; 12 Jul. 2017, ca. 10:00 hrs; Lal Rinsanga leg.; collected from a roadside) (Fig. 1).

Reproductive observations. On 10 Jul. 2019, the captive *B. reticulata* (MZMU 1424) began the oviposition of the first egg at ca. 8:30 hrs (room temperature 24.1 °C–25.5 °C; humidity: 84–89%); second egg at ca. 10:00 hrs; third egg at 10:58 hrs and completed ca. 13 minutes later (Fig. 2A–C); and then the fourth egg laid at 12:55. Oviposition resumed the next day with the fifth and sixth eggs laid at ca. 8:00 hrs–11:00 hrs, and finally the seventh egg laid at ca. 17:50 hrs (Fig. 2D). Eggs were whitish, soft, with a leathery texture and oblong shape. All of the seven eggs appeared fully viable at the initial stages and were incubated for several days at 26.5 °C–28 °C temperature and 85%–90%, humidity. Unfortunately eggs never hatched, either because of fungal infection or due to suboptimal temperature and humidity conditions. Thus, neither precise information on incubation temperature and humidity requirements, nor duration of incubation, time of hatching or neonate biometric data are available at this time.

On 11 Jul. 2019, the female was anaesthetized using 250mg/kg of 0.7% sodium bicarbonate buffered MS-222 (Tricaine Methanesulfonate) solution by intracoelomic injection, and then euthanized using a second intracoelomic injection of 0.1ml unbuffered 50% (v/v) MS-222 solution (see Conroy et al. 2009). The animal was dissected prior to preservation. Notably, we observed a second clutch of eggs (N = 8) in the oviduct (Fig. 3). The specimen was then fixed in 10% formalin, preserved in 70% ethanol, and catalogued as a voucher specimen in the Departmental Museum of Zoology, Mizoram University (MZMU 1424). A second specimen (MZMU 941) was also dissected and contained a seven egg clutch (Table 1 for the detailed egg measurements).

Discussion

The present work provides new distributional records for *Blythia reticulata* from the NE Indian state of Mizoram (Saitual, Khawzawl, Lungdai, Tanhril, and Tlungvel) in addition to the previously recorded sites i.e. Hmuifang, Sawleng, Sihphir, Durtlang, Sihhmui, and Aizawl (Vogel et al. 2017), and also expands the known elevational range of the species from the 949–1,040 m asl. zone up

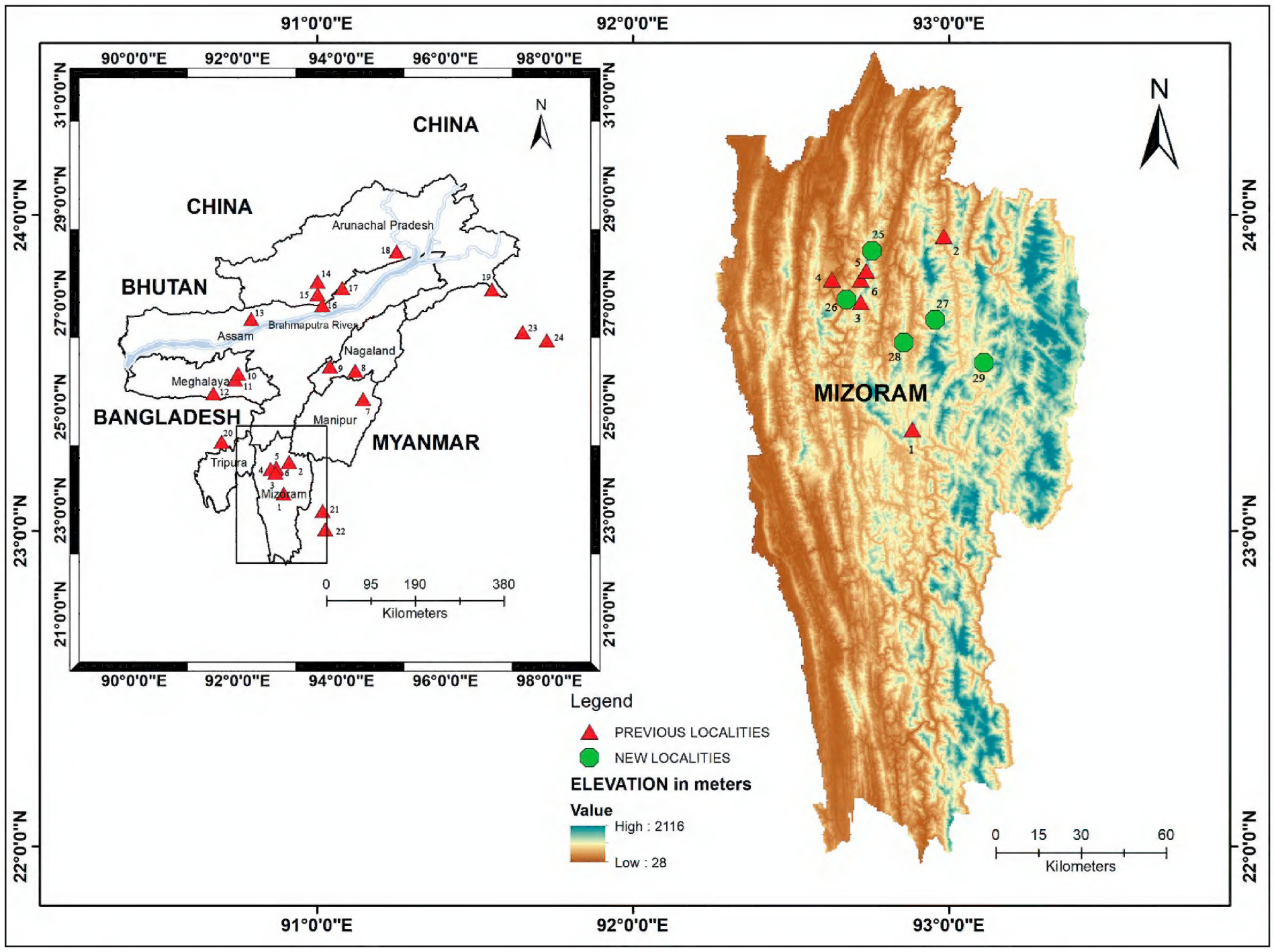

Figure 1. Map showing distributions of *Blythia reticulata*: (a) Map of NE India showing previous localities in red triangles (1. Hmuifang; 2.Sawleng; 3.Aizawl; 4.Sihhmui; 5.Sihphir; 6.Durtlang; 7.Ukhrul; 8.Kohima; 9.Samagooting; 10.Upper Shillong; 11.Shillong; 12.Cherrapunji; 13.Orang National Park; 14.Dafla Hill; 15.Itanagar; 16.Chessa; 17.Dejoo, of North Lakhimpur; 18.Renging, Janakmukh and Rotung; 19.Gandhigram; 20.Lawachara Reserve; 21 Fort White; 22.Hakka; 23.Sumprabum; 24.Htingnam) (see Vogel et al. 2017), and (b) Map of Mizoram showing previous localities in red triangles and new localities in green dots (25.Lungdai; 26.Tanhril; 27.Saitual; 28.Tlungvel; 29.Khawzawl).

Table 1. Eggs measurements (in mm) and weights (in g) of *Blythia reticulata*. All three clutches are at different stages of development, with only the first one representing sizes at parturition.

Eggs of MZMU 1424			Oviductal eggs of MZMU 1424		Eggs of MZMU 941	
Length	Width	Weight	Length	Width	Length	Width
17.9	9.3	0.46	5.9	2.9	15.9	6.6
11.1	9.4	0.38	6.1	3.4	14.4	8.2
16.2	9.8	0.42	6	3	14.9	7.2
17.1	10.7	0.48	5.3	2.9	14.5	6.8
17.4	8.2	0.42	6.1	3.4	13.8	7.6
15.4	10.3	0.42	6	3.1	16.6	7.1
20.7	9.4	0.47	5.8	3.4	16.8	6.9
			5.5	3		

to 1280 m asl. (see Whitaker and Captain 2008; Vogel et al. 2017). The specimens in this study were either collected from the side of the road, or were excavated from the ground. All individuals were collected from the microhabitats in the proximity of surface water, including streams, ponds and puddles. Because the species was encountered either in the morning or in the evening, we suggest it has likely a crepuscular pattern of natural activity. The climate pattern of Mizoram is moist tropical to moist sub-tropical with temperatures ranging between 18 °C–29 °C in summer, whereas in winter temperatures vary between 11 °C–24 °C; average annual rainfall in the region is about 2,540 mm (Geological Survey of India, 2011). The specimens were encountered between the onset and the end of the monsoon season (late February to October); gravid specimens were encountered during the wettest part of the monsoon season (May to July). Consequently, we argue that reproductive and breeding activities in *B. reticulata* coincide with the rainy season in this region.

Recent herpetological insights signified that the reproductive cycles of almost all snake species can be considered to some extent seasonal, with a pronounced absence of truly continuous patterns of reproduction in snakes (Almeida-Santos et al. 2006; Mathies 2011). The simultaneous presence of a second clutch of eggs at such a short time after oviposition, suggests that *B. reticulata* is also capable of multiple matings and/or multiple clutch-

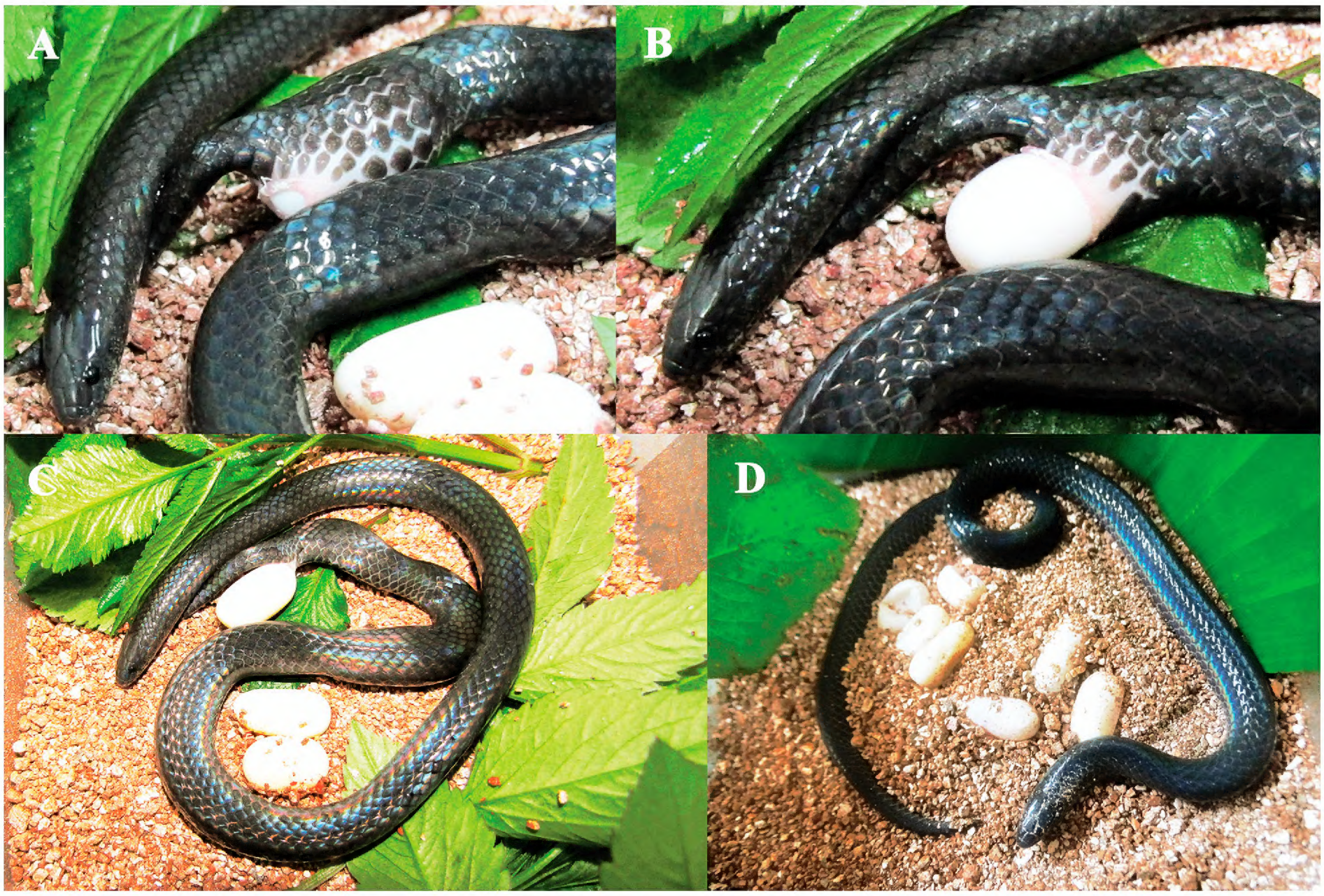

Figure 2. Observation on the oviposition of a female *Blythia reticulata*. **A–C** Initiation of the oviposition of third egg. **D** Female after termination of oviposition with a full, seven egg clutch.

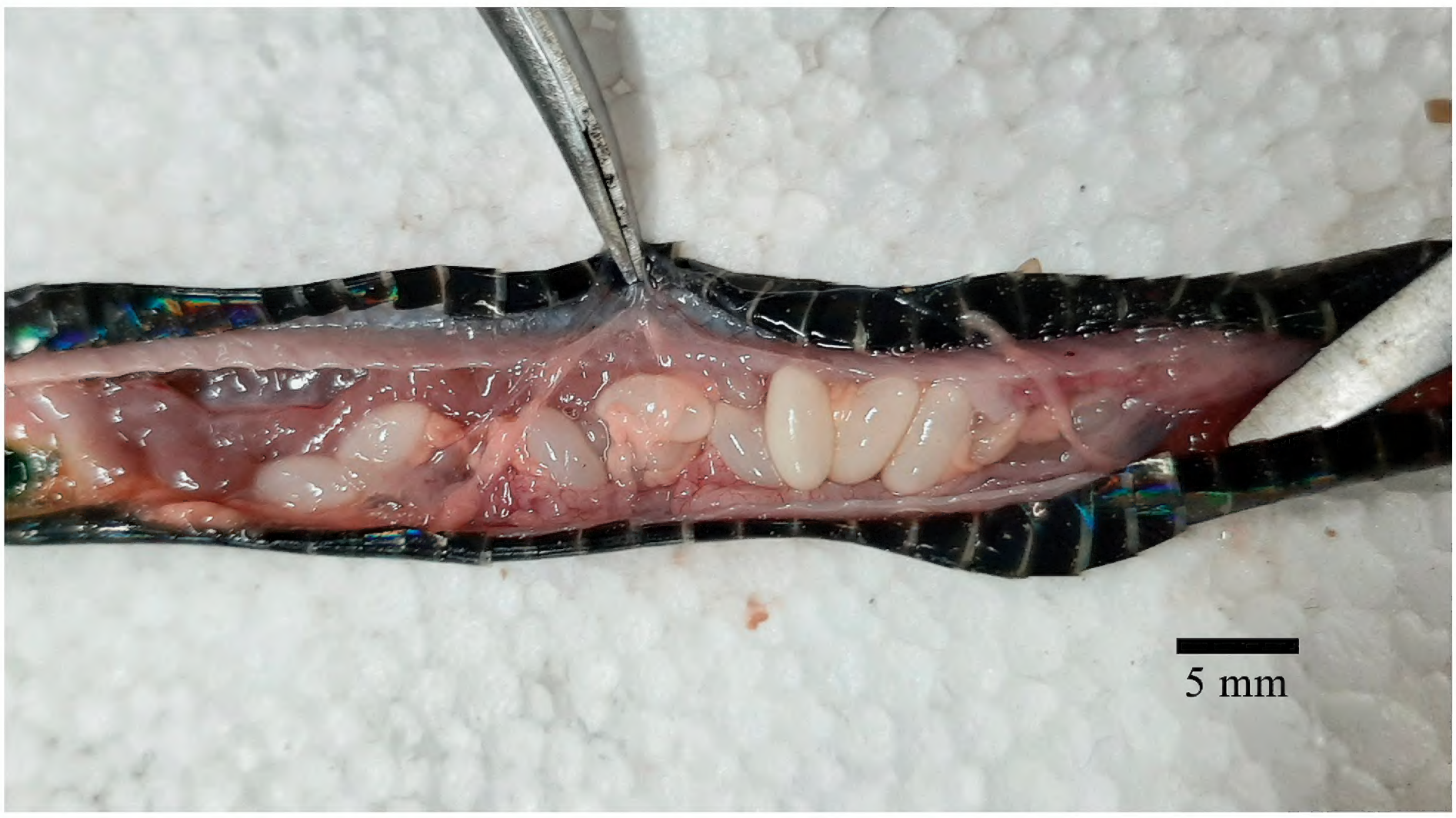

Figure 3. Oviductal eggs of *Blythia reticulata* (MZMU 1424).

es during a single reproductive period. This phenomenon appears to be rare in snakes, with only a handful of observations in some Brazilian snake species in the family Xenodontinae (Pinto and Fernandes 2004) and especially, *Philodryas nattereri* (Mesquita et al. 2011), and *Philodryas olfersii* (Mesquita et al. 2013). The present study

represents the first-ever documentation of oviposition in *B. reticulata*, with a maximum fecundity of 8 eggs vs. the 6 eggs previously reported by (Whitaker and Captain 2008). Although the female laid a total of 7 eggs, we considered maximum fecundity based on the number of eggs found in the oviduct by following Mesquita et al. (2013). According to Mathies (2011), data from specimens with eggs in the oviduct is the best metric for analysing reproductive cycle. Thus, the present publication serves as a novel contribution for this species, which because of its rarity and the secretive lifestyle, remains poorly known (Bassi et al. 2019). Further reproductive studies are needed to improve understanding and delimit the reproductive cycle of the snake species *B. reticulata*.

Acknowledgements

We are thankful to the Chief Wildlife Warden, Department of Environment, Forests and Climate Change, for issuing permission for herpetofaunal collection within the State. We also thank the Department of Science and Technology-Science and Engineering Research Board (DST-SERB), New Delhi, India for providing financial assistance. We would like to express our gratitude to the Head of the Department of Zoology, of Mizoram University for his continuous support throughout this work. The authors would like to also acknowledge Vishal Santra for proof-reading the manuscript. Lastly, our thanks were also extended to B.J. Hnamte, Lal Rinsanga, Michael Vanlalchhuana, Lal Muansanga, and Gospel Zothanmawia Hmar and Lalrengpuii Sailo for their assistance during the field surveys and laboratory work.

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